

WHAT IS CLAIMED IS:

1. An isolated or recombinant polypeptide comprising the amino acid sequence:

5 X₁ X₂ X₃ X₄ X₅ X₆ X₇ X₈ X₉ X₁₀ X₁₁

wherein X₁ is L, F, W, M, R, I, V, Y, K, or absent,

X₂ is Y, F, A, W, S or T,

X₃ is any amino acid,

X₄ is any amino acid,

10 X₅ is any amino acid,

X₆ is S, A, N, H or P,

X₇ is any amino acid,

X₈ is any amino acid,

X₉ is any amino acid or absent,

15 X₁₀ is N, G, L, S, M, P, N, A or absent, and

X₁₁ is L or absent,

wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

20 2. The isolated or recombinant polypeptide of claim 1, wherein X₁ is L, F, W, M, R or absent.

3. The isolated or recombinant polypeptide of claim 2, wherein X₁ is L, F or W.

25 4. The isolated or recombinant polypeptide of claim 1, wherein X₂ is Y, F, A.

5. The isolated or recombinant polypeptide of claim 1, wherein X₃ is R, T, S, H, D, G, A, L, K, A, N, Q or P.

30

6. The isolated or recombinant polypeptide of claim 5, wherein X_3 is R, T, S, H, D, G, A or L.

5 7. The isolated or recombinant polypeptide of claim 6, wherein X_3 is R, T, S or H.

8. The isolated or recombinant polypeptide of claim 1, wherein X_4 is S, T, G, A, L, R, I, M, V, P.

10 9. The isolated or recombinant polypeptide of claim 8, wherein X_4 is S, T, G, A, L, R.

15 10. The isolated or recombinant polypeptide of claim 9, wherein X_4 is S.

11. The isolated or recombinant polypeptide of claim 1, wherein X_5 is P, A, G, S or T.

20 12. The isolated or recombinant polypeptide of claim 1, wherein X_5 is P.

25 13. The isolated or recombinant polypeptide of claim 1, wherein X_6 is S, N, H, P, A, G or T.

14. The isolated or recombinant polypeptide of claim 13, wherein X_6 is S, N or H.

30 15. The isolated or recombinant polypeptide of claim 14, wherein X_6 is S.

16. The isolated or recombinant polypeptide of claim 1, wherein X₇ is M, F, Y, D, E, N, Q, H, G, I, L, V, A, P, N or W.

17. The isolated or recombinant polypeptide of claim 16, wherein X₇ is M, F, Y, D, E, N, Q or H.

18. The isolated or recombinant polypeptide of claim 17, wherein X₇ is M, F, Y, Q or H.

19. The isolated or recombinant polypeptide of claim 1, wherein X₈ is P, F, Y, W, L, G, M, D, E, N, Q, H, I, V, A or P.

20. The isolated or recombinant polypeptide of claim 19, wherein X₈ is P, F, Y or W.

21. The isolated or recombinant polypeptide of claim 20, wherein X₈ is Y.

22. The isolated or recombinant polypeptide of claim 1, wherein X₉ is E, G, L, S, M, P, N, D, A, T, P or absent.

23. The isolated or recombinant polypeptide of claim 1, wherein X₁₀ is absent.

24. The isolated or recombinant polypeptide of claim 1, wherein X₁₁ is absent.

25. The isolated or recombinant polypeptide of claim 1, wherein X₂ is Y, X₅ is P, and X₁₀ is N.

26. The isolated or recombinant polypeptide of claim 1, wherein X₃ is R, X₈ is P, and X₁₁ is L.

27. The isolated or recombinant polypeptide of claim 1, wherein X₄ is S, X₅ is P, X₆ is S, X₉ is E, X₁₀ is N and X₁₁ is L.

28. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises Y G G P G G G N.

29. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises R Y S L P P E L S N M.

30. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L A R S A S M P E A L.

31. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P S M P E N L.

32. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P A M P E N L.

33. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y R S P S F Y E N L.

34. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y R S P S Y Y E N L.

35. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y R S P S Y Y.

36. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P S Y P E N L, L Y R S P S Y F E N L, L Y R S P S Y Y E N L, or L Y R S P S Y W E N L.

5 37. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P S N P E N L, L Y R S P S N F E N L, L Y R S P S N Y E N L, or L Y R S P S N W E N L.

10 38. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y R S P S H P E N L, L Y R S P S H F E N L, L Y R S P S H Y E N L, L Y R S P S H W E N L, L Y S S P S M P E N L, L Y S S P S M F E N L, L Y S S P S M Y E N L, L Y S S P S M W E N L, L Y S S P S F P E N L, L Y S S P S F P E N L, L Y S S P S F F E N L, L Y S S P S F Y E N L, L Y S S P S F W E N L, L Y S S P S Y P E N L, L Y S S P S Y F E N L, L Y S S P S Y Y E N L, or L Y S S P S Y W E N L.

15 39. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y S S P S Q P E N L, L Y S S P S Q W E N L, L Y S S P S H P E N L, L Y S S P S H F E N L, L Y S S P S H Y E N L, L Y S S P S H W E N L, L Y T S P S M P E N L, L Y T S P S M F E N L, L Y T S P S M Y E N L, L Y T S P S M W E N L, L Y T S P S F P E N L, L Y T S P S F F E N L, L Y T S P S F Y E N L, L Y T S P S F W E N L, L Y T S P S Y P E N L, L Y T S P S Y F E N L, L Y T S P S Y Y E N L, or L Y T S P S Y W E N L.

20 40. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y T S P S N P E N L, L Y T S P S N F E N L, L Y T S P S N Y E N L or L Y T S P S N W E N L.

25 41. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y T S P S H P E N L, L Y T S P S H F E N L, L Y T S P S H Y E N L or L Y T S P S H W E N L.

42. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L Y H S P S Y P E N L, L Y H S P S Y F E N L, L Y H S P S Y Y E N L or L Y H S P S Y W E N L.

5 43. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L F T S P S Y P E N L, L F T S P S Y F E N L, L F T S P S Y Y E N L or L F T S P S Y W E N L.

10 44. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises F Y S S P S H P E N L, F Y S S P S H F E N L, F Y S S P S H Y E N L, F Y S S P S H W E N L, F Y T S P S M P E N L, F Y T S P S M F E N L, F Y T S P S M Y E N L, F Y T S P S M W E N L, F Y T S P S F P E N L, F Y T S P S F F E N L, F Y T S P S F Y E N L, F Y T S P S F W E N L, F Y T S P S Y P E N L, F Y T S P S Y F E N L, F Y T S P S Y Y E N L or F Y T S P S Y W E N L.

15 45. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y R S P S M P E N L, W Y R S P S M F E N L, W Y R S P S M Y E N L, W Y R S P S M W E N L, W Y R S P S F P E N L, W Y R S P S F F E N L, W Y R S P S F Y E N L, W Y R S P S F W E N L, W Y R S P S Y P E N L, W Y R S P S Y F E N L, W Y R S P S Y Y E N L or W Y R S P S Y W E N L.

20 46. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y T S P S M P E N L, W Y T S P S M F E N L, W Y T S P S M Y E N L, W Y T S P S M W E N L, W Y T S P S F P E N L, W Y T S P S F F E N L, W Y T S P S F Y E N L, W Y T S P S F W E N L, W Y T S P S Y P E N L, W Y T S P S Y F E N L, W Y T S P S Y Y E N L or W Y T S P S Y W E N L.

25 47. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises W Y T S P S H P E N L, W Y T S P S H F E N L, W Y T S P S H Y E N L or W Y T S P S H W E N L.

48. The isolated or recombinant polypeptide of claim 1, wherein the amino acid sequence comprises L K R S P S M P E N L, L Y I S P S M P E N L or L Y R S P S M V E N L.

5 49. The isolated or recombinant polypeptide of claim 1, wherein the cell is a mammalian cell.

50. The isolated or recombinant polypeptide of claim 49, wherein the cell is a human cell.

10 51. The isolated or recombinant polypeptide of claim 1, further comprising a cell membrane permeant.

15 52. The isolated or recombinant polypeptide of claim 51, wherein the cell membrane permeant comprises a polypeptide.

53. The isolated or recombinant polypeptide of claim 52, wherein the polypeptide comprises a TAT protein transduction domain.

20 54. The isolated or recombinant polypeptide of claim 53, wherein the TAT protein transduction domain is Y G R K K R R Q R R R.

25 55. The isolated or recombinant polypeptide of claim 51, wherein the cell membrane permeant comprises a lipid.

56. The isolated or recombinant polypeptide of claim 55, wherein the cell membrane permeant comprises a liposome.

30 57. A chimeric polypeptide comprising a first domain comprising a polypeptide as set forth in claim 1 and a second domain comprising a cell membrane

permeant, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

58. The chimeric polypeptide of claim 57, wherein the polypeptide is a recombinant fusion protein.

59. An isolated or recombinant nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

60. An expression vector comprising a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

61. A cell comprising a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint.

62. The cell of claim 61, wherein the cell is a bacterial, a yeast, an insect, or a mammalian cell.

63. A pharmaceutical composition comprising a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint, a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint, an expression vector comprising a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint, or

a cell comprising a nucleic acid encoding a polypeptide as set forth in claim 1 or claim 57, wherein the polypeptide when administered to or expressed in a cell disrupts the G2 cell cycle arrest checkpoint; and,

a pharmaceutically acceptable excipient.

5

64. The pharmaceutical composition of claim 63 comprising a liposome.

65. A method for inhibiting a the activity of a Chk1 kinase or a Chk2 kinase comprising contacting the kinase with a polypeptide as set forth in claim 1 or claim 57 or a pharmaceutical composition as set forth in claim 63, in an amount sufficient to inhibit the activity of the Chk1 or Chk2 kinase.

10

15

66. A method for disrupting a cell G2 cell cycle arrest checkpoint comprising contacting the cell with a polypeptide as set forth in claim 1 or claim 57 or a pharmaceutical composition as set forth in claim 63, in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint.

20

67. A method for sensitizing a cell to a DNA damaging agent comprising contacting the cell with a polypeptide as set forth in claim 1 or claim 57 or a pharmaceutical composition as set forth in claim 63, in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint, thereby sensitizing the cell to the DNA damaging agent.

25

68. The method of claim 67, wherein the cell is a human cell.

69. The method of claim 67, wherein the cell is a cancer cell.

30

70. A method for selectively sensitizing a cell with an impaired G1 cell cycle arrest checkpoint to a DNA damaging agent comprising contacting the cell with a polypeptide as set forth in claim 1 or claim 57 or a pharmaceutical composition as set forth in claim 63, in an amount sufficient to disrupt the G2 cell cycle arrest checkpoint, thereby sensitizing the cell to the DNA damaging agent.

71. The method of claim 70, wherein the cell is a cancer cell.

72. A method for inducing apoptosis in a cancer cell in an individual
5 comprising a administering a polypeptide as set forth in claim 1 or claim 57 or a
pharmaceutical composition as set forth in claim 63, in an amount sufficient to disrupt the G2
cell cycle arrest checkpoint in the cancer cell, thereby sensitizing the cancer cell to a DNA
damaging agent, and administering a DNA damaging agent.

10 73. The method of claim 72, wherein the DNA damaging agent is 5-
fluorouracil (5-FU), rebeccamycin, adriamycin, bleomycin, cisplatin, hyperthermia, UV
irradiation or gamma-irradiation.

15 74. A method for screening for compounds capable of modulating the
activity of a Chk1 kinase or a Chk2 kinase comprising the following steps
(a) providing a test compound;
(b) providing a Chk1 kinase or a Chk2 kinase;
(c) providing a polypeptide as set forth in claim 1 or claim 57, wherein the
20 polypeptide binds to the Chk1 kinase or the Chk2 kinase; and
(d) contacting the test compound with the kinase and the polypeptide and
measuring the ability of the test compound to prevent binding of the polypeptide to the
kinase.

25 75. A method for screening for compounds capable of modulating the
activity of a Chk1 kinase or a Chk2 kinase comprising the following steps
(a) providing a test compound;
(b) providing a Chk1 kinase or a Chk2 kinase;
(c) providing a polypeptide as set forth in claim 1 or claim 57, wherein the
30 polypeptide is phosphorylated by the Chk1 kinase or the Chk2 kinase; and

(d) contacting the test compound with the kinase and the polypeptide and measuring the ability of the test compound to inhibit or abrogate phosphorylation of the polypeptide by the kinase.

5 76. The method of claim 75 further comprising providing a full length human Cdc25C.

77. The method of claim 75, wherein the polypeptide of step (c) comprises amino acid residue serine 216 of human Cdc25C.

10 78. The method of claim 77, wherein the polypeptide is a peptide comprising from about amino acid residue 200 to about amino acid residue 250 of human Cdc25C.

15 79. The method of claim 74 or claim 75, wherein the polypeptide of step (c) further comprises glutathione-S-transferase.

80. The method of claim 74 or claim 75, wherein the polypeptide of step (c) is immobilized.

20 81. A method for screening for compounds capable of specifically inhibiting or abrogating the G2 cell cycle arrest checkpoint comprising the following steps

(a) providing a test compound and a polypeptide as set forth in claim 1 or claim 57;

25 (b) providing a G1 checkpoint impaired cell;
(c) contacting the cell of step (b) with the test compound or the polypeptide of step (a) and a DNA damaging treatment or an M phase checkpoint activator; and

(d) measuring the amount of DNA in the cells after the contacting of step (c) to determine if the test compound has inhibited or abrogated the G2 cell cycle arrest
30 checkpoint, wherein the polypeptide of step (a) acts as a G2-checkpoint-inhibiting positive control.

82. The method of claim 81, wherein the amount of DNA is measured using propidium iodide and FACS analysis.

5 83. The method of claim 81, wherein the amount of DNA is measured after about 10 to about 72 hours after the contacting of step (c).

84. The method of claim 81, wherein the cell is contacted with an M phase checkpoint activator and a test compound or a polypeptide of step (a), wherein a test
10 compound that has not inhibited or abrogated the arrest at the M phase checkpoint of the cell cycle after contacting the cell with an M phase activator is a specific inhibitor of the G2 cell cycle arrest checkpoint.

85. The method of claim 84, wherein the M phase checkpoint activator is
15 colchicine or nocodazole.

86. The method of claim 81, wherein the DNA damaging treatment is 5-fluorouracil (5-FU), rebeccamycin, adriamycin, bleomycin, cisplatin, hyperthermia, UV irradiation or gamma-irradiation.